THE REVERSION OF HÆMOLYSIS. By R. BRINKMAN AND A. v. SZENT-GYÖRGYI.

(From the Physiological Institute, Groningen.)

By the term reversion of hæmolysis we understand the reappearance of apparently normal red corpuscles in hæmolysed blood, so that normal blood which had been completely hæmolysed, is reversed for the greater part into normal blood once more. This process can easily be observed if one takes care to regulate the hæmolytic influence in such a way, that the occurring lysis is only a hæmoglobinolysis and not a stromatolysis. In most of the hæmolytic processes, including the biological ones, the effect of not too large concentrations of hæmolytics is the production of spherical corpuscles, followed by a rather sudden loss of the pigment. The hæmoglobin does not slowly diffuse out of the cell, but comes out rapidly. If the stroma is preserved in a more or less undamaged condition, it is possible to bring the hæmoglobin back to the stromata, so that all stromata are suddenly changed once more to apparently quite normal corpuscles. The "re-adsorption" of hæmoglobin takes place with the same velocity as the chromolysis.

The phenomenon may be observed in the following way. 10 c.c. of defibrinated blood are thoroughly shaken with ·02 c.c. of a pure higher fatty acid; we always used linolenic acid, because of the solubility of its Ca-soaps. The blood is then placed in the water bath at 37° and the electrical conductivity measured at short intervals. When the said concentration of linolenic acid is given, a steady increase of the resistance is always noted; when after 1–3 hours hæmolysis has become complete, the resistance has increased considerably and remains as high for 1–2 days. But if some more linolenic acid is added, the resistance suddenly decreases to nearly the serum value, and in this blood no stromata are present and reversion is not possible.

The following table gives the conductivity of the different sorts of hæmolysed blood, at 37°.

	$\lambda . 10^4$
Normal pig's blood	63
Normal pig's serum	137
The same blood hæmolysed with ·02 c.c. of linolenic acid in 2 hrs.	39
The same blood hæmolysed with ·04 c.c. of linolenic acid	125
The same blood laked by mechanical hæmolysis[1]	138

[1] By the method of D. J. de Waard. This Journ. 57. p. 195. 1923.

It is easily demonstrable, that in blood, laked by an appropriate concentration of linolenic acid, with a high electrical resistance, the stromata are all present, not very much swollen and not agglutinated. This may be seen by looking at the blood when it is still streaming under the coverslip, or in a drop of hæmolysed blood, mixed with a small amount of China ink. The presence of stromata may, of course, also be proved by fixation with methyl alcohol and staining with methyl violet.

Fig. 1 is a microphotograph of hæmolysed pig's blood which was

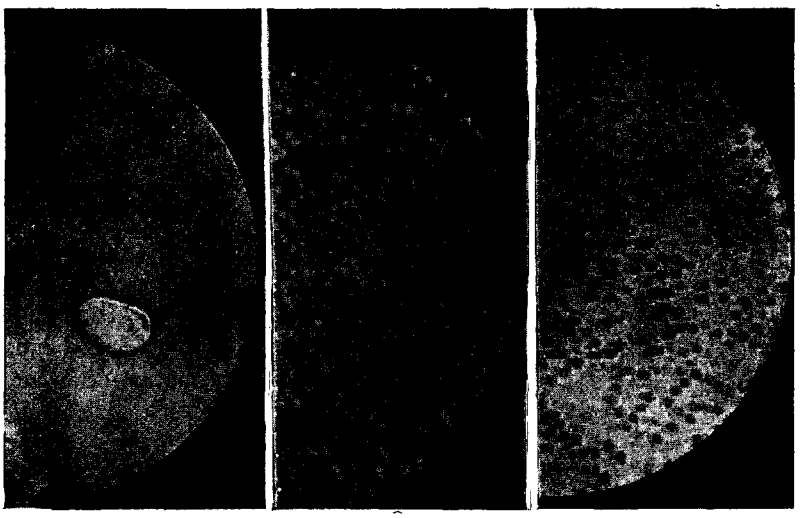

Fig. 1. Fig. 2. Fig. 3.

somewhat diluted with serum; the contrast with the air bubble shows the hæmoglobin colour; the stromata are invisible. Fig. 2 is a microphotograph of the same blood, mixed with a trace of China ink; numerous stromata are to be seen as white spaces.

If now a small amount of isotonic saline is added to this completely hæmolysed blood, the fluid becomes clouded and bright red like normal blood. Microscopically the corpuscles have reappeared with their greenish colour; no hæmoglobin is to be seen between the cells although in the laked blood the yellow colour is observed quite distinctly between the ghosts. The reversion can directly be seen microscopically if a small drop of the laked blood on a slide is brought under the coverslip and a small amount of isotonic salt solution is run in from this side. Where the saline streams into the laked blood, the corpuscles suddenly reappear. Fig. 3

is a microphotograph of the same laked blood as that in Figs. 1 and 2, after the addition of a little salt solution under the coverglass. The stromata have got back their hæmoglobin. No colour was visible between them. The form of the corpuscles may be biconcave if they are floating but is always spherical if they come into contact with the glass. This is not characteristic of "reversed corpuscles" but of red cells in salt solution in contact with the slide(1).

It might be supposed that the transparency of laked blood should not be ascribed to the solution of hæmoglobin in the cell-surrounding medium, but to a change of the refraction of the red cells, or to a direct complete contact of all swollen corpuscles. Though it is distinctly to be seen in a fresh film of laked blood that all stromata have retained their form and are floating freely in a yellow solution of hæmoglobin (see Figs. 1 to 3) we have brought further evidence by determining the refractive indices of laked and of reversed blood. These showed that all hæmoglobin had left the stromata and that in reversion 50–60 p.c. of the hæmoglobin was no longer in solution but must have gone back to the cells[1].

If the laked blood is diluted with a drop of a 27·8 p.c. (by weight) solution of cane sugar which has the same refraction as laked blood, the reversion is very distinct and measurable by refraction-differences. Moreover we have studied the phenomenon in corpuscles which were five times washed in a balanced salt solution in order to remove the serum. If this suspension of red cells in about the same concentration as in normal blood is laked carefully by ·01 c.c. of linolenic acid to 10 c.c. of suspension at 37° C., the ghosts can be seen distinctly and the reversion, caused by addition of neutral isotonic phosphate-solution[2], is nearly complete. If this reversed suspension is centrifugalised and washed once with a small amount of salt solution, the hæmoglobin nearly completely disappears from the solution and passes back to the cells. Complete re-adsorption of the pigment to the stromata, cannot, however, be obtained in this way. There always are some corpuscles which lose their hæmoglobin for the second time. If reversion is made in serum, the second hæmolysis appears in a short time. The more completely the serum is removed, the longer the corpuscles will remain in the reversed state. If to the reversed washed corpuscles in salt solution, which will retain their hæmoglobin for hours, a small amount of serum is added, the chromolysis occurs at once. When this second hæmolysis is observed

[1] We desire to thank Dr de Waard for the determination of refraction indices.

[2] 900 c.c. of 2·5 p.c. Na_2HPO_4 7 aq. + 100 c.c. of 1·7 p.c. KH_2PO_4 + 50 aq.

microscopically, it is seen that hæmoglobin again comes off suddenly, not slowly.

Nearly complete and constant re-adsorption of the hæmoglobin to the ghosts can be observed in blood, laked by freezing and thawing, and by hypotonia. In blood, laked by careful freezing and thawing, the electrical resistance is high and the ghosts are visible. Addition of salt solution brings the hæmoglobin back to the stromata, so that the cells can be separated by centrifuge and washed. The hæmolysis by hypotonia was made by the removal of the serum and addition of a phosphate solution with a concentration slightly less than half of isotonic. The mixture was centrifuged so that all resistant corpuscles were removed; in the completely transparent hæmolysed fluid the ghosts could be seen floating in the clear yellow hæmoglobin solution. Addition of isotonic phosphate solution brings the corpuscles back; they can be centrifuged and washed and do not lose their pigment again. Addition of solid NaCl to restore isotonicity brings about the same reversion.

A rapid way of demonstrating the phenomena is the following. The defibrinated blood is centrifuged, the serum removed and the corpuscles washed in isotonic phosphate-solution. Hæmolysis is made by slowly adding 1·6 c.c. of distilled water to 1 c.c. of blood-corpuscles-suspension. Reversion is made by addition of 2·6 c.c. of $\frac{5}{3}$ hypertonic phosphate-solution (2·25 gr. Na_2HPO_4 7 aq. + 0·17 gr.) KH_2PO_4 in 60 c.c. water.

We have not tried the possibility of reversion in other forms of hæmolysis but believe that reversion is possible if hæmolysis is only chromolysis, as it is nearly always when the hæmolytic influence is not too strong[1].

We do not know if the phenomenon has any biological importance but think that it points very clearly to the fact that hæmoglobin cannot be present in the red cells as a droplet of solution surrounded by a vesicle, but that for the greater part it must be attached to the surface of the stromata. The always observable fact that the electrical resistance of the blood is not decreased after chromolysis makes it very improbable that in laking the very big hæmoglobin molecule passes through cell-surfaces which remain impermeable to crystalloid ions. The statement of Bürker(2) that the corpuscular amount of hæmoglobin is proportional to its surface and not to its volume, also brings evidence that most of the red pigment must be concentrated in the surface of the blood cells.

It would be very curious if a phenomenon which is so easily observed

[1] Reversion is not possible in blood, laked by amboceptor + complement. If amboceptor is added to hæmolysed blood, reversion is still possible, but reversibility disappears if amboceptor + complement are added to hæmolysed blood.

as the reversion of hæmolysis, especially that caused by hypotonia, had not formerly been noticed. Spiro is said by Rohonyi(3) to have described in 1897 the passing back of hæmoglobin and stromata on adding salt solution after hypotonia hæmolysis. Rohonyi more or less confirmed Spiro's results, but stated that the "reversal" occurred only in cells which were incompletely hæmolysed, though they were swollen to invisibility. On adding salt these cells, he said, shrank and they and the hæmoglobin in them again became visible. The phenomenon was independently observed by Adair, Barcroft and Bock(4) in blood, laked by dialysis, but apparently they considered it to be due to the assumption and subsequent loss of water by the cells, and not to dissolved extracellular hæmoglobin rejoining the stroma.

It may be mentioned that an entirely different form of "reversal" is described by Rohonyi. When he completely hæmolysed the blood by water or saponin, he found that on adding a certain amount of a substance precipitating protein, the precipitate consisted entirely of cells, containing a brown hæmoglobin derivate. We do not think that this experiment may be called a true reversion of the hæmolytic process.

REFERENCES.

(1) Brinkman and van Dam. Biochem. Ztsch. 108. p. 52. 1920.
(2) Bürker. Pflüger's Arch. 195. p. 516. 1922.
(3) Rohonyi. Kolloidchem. Beihefte 8. p. 391. 1916.
(4) Adair, Barcroft and Bock. Journ. Physiol. 55. p. 332. 1921